

HISTOLOGICAL AND FUNCTIONAL COMPARISON IN THE MOUSE OVARY UNDER NORMAL  
CONDITIONS AND IN HYPERVITAMINOSIS A

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Vitamin A has recently attracted the attention of clinicians because of its specific and nonspecific therapeutic action in certain diseases. Retinol and its synthetic analogs also have the property of inhibiting growth of tumors, chiefly those of epithelial origin [1, 2, 4]. The doses of vitamin A preparations used are frequently some ten times higher than the physiological requirements. Accordingly, the study of the action of large doses of retinol on various systems of the body is an urgent matter. There are data in the literature on the state of the adrenals, testes, salivary glands, and various other organs in hypervitaminosis A [3, 5-8]. However, the effect of high doses of retinol on the female reproductive system has virtually not been studied.

Previously the writers showed changes in the dynamics of the estrous cycle in mice with hypervitaminosis A (80,000 IU). The normal sex cycle in female rodents consists of consecutive phases: proestrus, estrus, metestrus, and diestrus, with a mean duration of 0.5, 2.8, 1.85, and 2.7 days respectively. In hypervitaminosis A these cyclic changes cease.

The object of this investigation was to study the dynamics of changes in the follicular apparatus and its derivatives in different stages of the estrous cycle in the ovaries of intact mice and to describe the morphology of the ovaries in animals with hypervitaminosis A (80,000 IU).

#### EXPERIMENTAL METHOD

Experiments were carried out on 56 adult mature female CBA mice. A 3.44% oily solution of retinol acetate was used as the retinoid. The preparation was given by gastric tube in a dose of 8000 IU daily for 10 days. Control animals were given the neutral solvent, namely soy oil, by the same scheme. On the 11th day of the experiment the mice were decapitated. The ovaries were fixed in Carnoy's fluid. Paraffin sections were stained with hematoxylin and eosin.

The mean number of follicles of different degrees of maturity per unit area was counted in the stained sections: primordial, growing follicles with two or three layers of follicular cells, medium-sized, with four or five layers of follicular cells, large with six layers of cells of follicular epithelium or more and with signs of formation or the presence of a follicular cavity, and also atretic follicles and corpora lutea. The mean dimensions of the structures mentioned above were determined by means of an ocular micrometer. The morphometric analysis was undertaken under standard magnification of the microscope. The numerical results were subjected to statistical analysis.

#### EXPERIMENTAL RESULTS

The stage of proestrus in ovaries of intact mice is characterized by growth of follicles, the formation of Graafian follicles, and the commencement of ovulation. Against the background of small and medium-sized follicles, certain follicles containing eight layers of follicular cells or more and a clearly defined cavity were distinguished by their size (up to 300  $\mu$  in diameter). Lutein tissue was represented by old corpora lutea, remaining from previ-

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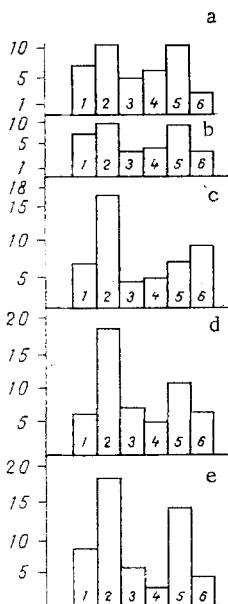


Fig. 1

Fig. 1. Distribution of principal structural elements of the mouse ovary per unit area of section: a) proestrus (control), b) estrus (control), c) metestrus (control), d) diestrus (control), e) hypervitaminosis A (80,000 IU). 1) Primordial, 2) growing, 3) medium-sized, 4) large follicles; 5) corpora atretica, 6) corpora lutea.

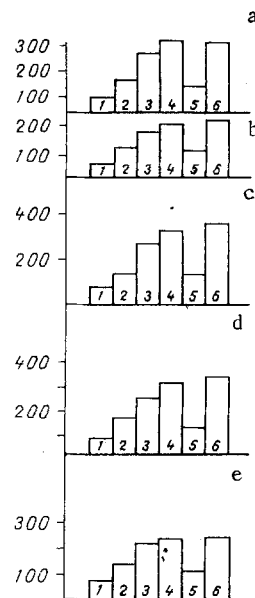


Fig. 2

Fig. 2. Dimensions of principal structural elements of mouse ovaries (in  $\mu$ ). Legend as in Fig. 1.

ous estrous cycles. Clear evidence of luteolysis could be seen in them. Corpora atretica were few in number ( $9.3 \pm 0.38$  per section) but were relatively small in size (Figs. 1a and 2a).

In the stage of estrus the corpora lutea formed in the preceding stage underwent development. They grew to a considerable size ( $288.33 \pm 12 \mu$ ). Most follicles were primordial or small, growing follicles. Larger follicles consisting of several layers and formed by stratified epithelium but, as a rule, without cavities or with only the initial signs of cavity formation also were found. The number of corpora atretica was a little smaller than in the phase of proestrus (Figs. 1b and 2b).

The stage of metestrus was characterized by completion of formation of corpora lutea with a lumen (Figs. 1c and 2c). They attained their largest size (diameter  $347.72 \pm 13.52 \mu$ ). The follicles as a whole were comparatively small, with two or three layers of epithelial cells ( $15.41 \pm 0.54$  per section). Medium-sized and large follicles were much less frequently found ( $3.0 \pm 0.15$  and  $3.09 \pm 0.04$  per section, respectively). Corpora atretica were less numerous ( $6.38 \pm 0.19$  per section), but were rather larger in size (diameter  $126.19 \pm 15.49 \mu$ ).

In the stage of diestrus, against the background of numerous small ( $18.06 \pm 0.29$  per section) and medium-sized ( $6.17 \pm 0.17$  per section) growing follicles, the number of corpora atretica ( $10.75 \pm 0.29$  per section) and their size (diameter  $128.78 \pm 5.11 \mu$ ) attained their highest values — the peak of follicular atresia (Figs. 1d and 2d). Processes of luteolysis began in the corpora lutea, accompanied by a relative decrease in their size and frequency ( $5.84 \pm 0.01$  per section).

In hypervitaminosis A (80,000 IU) cyclic changes were not found in the ovaries (Figs. 1e and 2e). Considerable structural changes were observed in the ovaries. Mass atresia of the follicles, with an obliterative type of course, predominated. Corpora atretica were smaller (diameter  $102 \pm 3.3 \mu$ ) and were observed more frequently ( $12.75 \pm 0.51$  per section) than in the intact animals. The number of growing follicles, especially medium-sized, was reduced

( $2.13 \pm 0.106$  per section) and large follicles with cavities were virtually absent. Ovulation did not take place and no corpora lutea were formed. Old corpora lutea left from previous estrous cycles were few in number ( $3.947 \pm 0.195$  per section). Meanwhile the development of interstitial tissue took place, merging diffusely with elements of the stroma of the ovaries.

Under normal conditions regular cyclic histological and functional changes, a component part of the estrous cycle, thus take place in the ovaries of normal female mice.

In hypervitaminosis A (80,000 IU) no estrous cycle was present in female mice. Growth of the follicles in the ovaries was disturbed and atresia proceeded intensively. As a result the follicles did not attain the final stage of their development, ovulation ceased, and no corpora lutea formed.

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